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Teneurin-2 (TENM2) deficiency induces UCP1 expression in differentiating human fat cells

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Abbreviations

adip adipocyte, ACAA1 acetyl-CoA acyltransferase 1, ACACA acetyl-CoA carboxylase alpha, ACOX1 acyl-CoA oxidase 1, CIDEA Cell Death-Inducing DFFA-Like Effector A, CIDEC cell death inducing DFFA like effector c, CPT1B Carnitine Palmitoyltransferase 1B, dn deep neck, ELOVL3 ELOVL fatty acid elongase 3, FCCP Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone, LHX8 LIM Homeobox 8, MYF5 Myogenic Factor 5, OCR oxygen consumption rate, PGC1a Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PPARg Peroxisome Proliferator Activated Receptor Gamma, PRDM16 PR domain containing 16, pre preadipocytes, sc subcutaneous, SGBS Simpson-Golabi-

Behmel syndrome, TMEM26 transmembrane protein 26, TENM2 teneurin-2, TCAP teneurin c-terminal associated peptide, ZIC1 Zic Family Member 1

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Disclosure

The authors declare no conflict of interest.

Abstract

Under certain conditions UCP1 expressing adipocytes arise in white adipose tissue depots of both mice and humans. It is still not fully understood whether these cells differentiate *de novo* from specific progenitor cells or if they transdifferentiate from mature white adipocytes. Performing expression pattern analysis comparing adipocyte progenitor cells from deep and subcutaneous neck adipose tissue, we recently identified teneurin-2 (TENM2) enriched in white adipocyte progenitor cells. Here we tested whether TENM2 deficiency in adipocyte progenitor cells would lead to a brown adipocyte phenotype. By targeting TENM2 in SGBS preadipocytes using siRNA, we demonstrate that TENM2 knockdown induces both UCP1 mRNA and protein expression upon adipogenic differentiation without affecting mitochondrial mass. Furthermore, TENM2 knockdown in human SGBS adipocytes resulted in increased basal and leak mitochondrial respiration.

In line with our previous observation these data suggest that TENM2 deficiency in human adipocyte precursors leads to induction of brown adipocyte marker genes upon adipogenic differentiation.

Keywords

brown adipose tissue, TENM2, adipogenesis, obesity

Introduction

Adipose tissue can be assigned to two clearly distinct subtypes, mainly based on its functional properties. While white adipose tissue (WAT) is the major organ for energy storage, brown adipose tissue (BAT) utilizes energy sources such as fatty acids and glucose to produce heat in a process called non-shivering thermogenesis (Tews and Wabitsch, 2011). In contrast to earlier observations, it is currently well accepted that BAT in humans is functionally active not only in neonates, but also in adults (Tews and Wabitsch, 2011). Interestingly, brown-like adipocytes can emerge in white adipose tissue depots upon chronic cold exposure or beta-adrenergic treatment, a process usually termed "browning" or "britening" (Bartelt and Heeren, 2014). As a matter of debate, these adipocytes can either differentiate *de novo* from a distinct precursor pool (Wang et al., 2013; Wu et al., 2012) or convert from preexisting white adipocytes (Rosenwald et al., 2013).

Due to the exceptionally high energy demand of BAT, the generation of brown adipocytes is of interest as a potential target for obesity treatment (Cypess and Kahn, 2010). Thus, the identification of genes involved in the process of browning will help to develop new strategies for obesity treatment. In an earlier study, we isolated progenitor cells from paired human adipose tissue samples derived from either the subcutaneous or the deeper-lying neck region (Tews et al., 2014). We found that upon adipogenic differentiation *ex vivo*, the cells isolated from the deep neck region resembled the phenotype of brown adipocytes with expression of UCP1 and other marker genes of brown adipocytes such as PRDM16 and LHX8 along with functional alterations in mitochondrial respiration, whereas cells from the subcutaneous region resembled a white phenotype (Tews et al., 2014). To identify potential target genes promoting or inhibiting brown adipocyte generation, we had analyzed gene expression patterns in progenitor cells isolated from subcutaneous white and deep neck, presumably brown adipose tissue depots. Herein, TENM2 was highly enriched (approx. 7 fold) in white compared to brown adipocyte progenitor cells. (Tews et al., 2014).

TENM2 belongs to the family of teneurins, which are large, type II cell surface proteins with a single transmembrane domain (Tucker et al., 2007). TENM2 is predominantly expressed in the brain, where it is involved in regulating synaptic connections, most likely by binding to latrophilin, a neuronal G-protein coupled receptor (Mosca, 2015). By using fusion proteins and reporter assays, it has been demonstrated that TENM2 is proteolytically cleaved at different sites on both sides of the plasma membrane thus releasing extracellular as well as intracellular domains (Mosca, 2015; Vysokov et al., 2016). The intracellular domain may thereby act as a inhibitor of the transcription factor ZIC1 (Bagutti et al., 2003). Since ZIC1 is used as a marker gene to identify classical brown adipocytes (Jespersen et al., 2013; Lidell et al., 2013), we decided to study the role of TENM2 in white and brown adipogenesis.

Material and Methods

Human primary adipose tissue samples TENM2 expression was analyzed in adipose tissue biopsies taken from patients undergoing neck surgery (Tews et al., 2014). The study was approved by the ethical committee of Ulm University (320/14) and all patients gave written informed consent. Stromal-vascular cells of either subcutaneous (sc) or deep neck (dn) adipose tissue had been isolated by collagenase digestion and were cultivated in cell culture dishes until subconfluence before harvesting RNA. The subcutaneous-derived progenitor cells differentiated into a white phenotype, whereas cells from the deep neck resembled the phenotype of brown adipocytes upon *ex vivo* adipogenic differentiation (Tews et al., 2014).

Cell culture and siRNA transfection Human SGBS preadipocytes were maintained and differentiated as described before (Fischer-Posovszky et al., 2008; Wabitsch et al., 2001). At defined time points during adipogenic differentiation, cells were fixed in 4% paraformaldehyde and subsequently stained with OilRed O to visualize lipid droplet formation. Two days before starting adipogenic differentiation, SGBS preadipocytes were treated with each 50 nM control siRNA or siRNA directed against human TENM2 (Riboxx, Radebeul, Germany, table S1) in the presence of Lipofectamine 2000 (ThermoFisher Scientific, Waltham, USA). Knockdown efficiency was controlled by qRT-PCR. At day 14 of adipogenic differentiation, cells were analyzed.

Expression analysis After isolation with the Direct-Zol RNA kit (Zymo Research, Irvine, USA), 1 µg of total RNA was transcribed into cDNA using SuperScript II reverse transcriptase (ThermoFisher Scientific). Relative expression of target genes was analyzed by quantitative real-time PCR using the ssoAdvanced Universal SYBR Green Supermix on a CFX Touch Real Time PCR Detection System (BioRad, Munich, Germany). Expression values were calculated using the ddCt method with hypoxanthine-guanine phosphoribosyltransferase (HPRT) as a reference gene. Cellular protein was extracted and protein expression was analyzed by Western blot analysis as described before (Tews et al., 2013). The following antibodies were used: rabbit anti-UCP1 (ab10938, Abcam, Cambridge, UK),

mouse anti-beta-actin (A5441, Sigma-Aldrich, St. Louis, USA), total OXPHOS human WB antibody kit (Abcam, #ab110411), mouse anti-alpha-tubulin (CP06, Millipore, Billerica, USA).

Mitochondrial quantification Citric acid synthase activity was assayed as a measure for mitochondrial content and activity as described before (Tews et al., 2013). In brief, 5 µg total protein was added to reaction buffer (100 mM Tris HCl pH 8.1, 100 µM 5,5' dithiobis-2-nitrobenzoic acid (DTNB), 300 µM acetyl-CoA, 0.1 % Triton X 100) and specific activity was determined by measuring conversion of DNTB to 2-nitro-5-benzoic acid (TNB) at a wavelength of 405 nm after adding 500 µM oxaloacetate as substrate.

Oxygen consumption measurements Cells were plated in 96-well cell culture microplates (Xe96, Agilent Technologies, Santa Clara, USA) and were differentiated into adipocytes. One day before measurement, cells were cultivated in insulin-free medium. On the day of measurement, cells were incubated for 1 h in unbuffered DMEM medium containing 10 mM glucose and 1% bovine serum albumin. Oxygen consumption was measured by a whole cell respirometer (Seahorse XFe96 Flux Analyzer, Agilent Technologies). In order to mimic thermogenic conditions, cells were treated with 0.5 mM dibutyryl cyclic adenosinmonophosphate (cAMP). Uncoupled respiration was profiled by injecting 5 µM oligomycin, and full respiration capacity was determined by injecting 2 µM carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) into the wells. Non-mitochondrial respiration was determined by adding antimycin A and rotenone (0.5 µM each). Oxygen consumption rates (OCR) were determined by plotting the partial oxygen pressure against time which were normalized to adipocyte number.

Statistics If not otherwise stated, data from three independent triplicate experiments were expressed as mean +/- standard error of means (SEM). For statistical comparison, Analysis of variants test (ANOVA) or t-test was used as indicated in the figure legends. A p value $p<0.05$ was considered

statistically significant. For the analysis, GraphPad Prism version 6.07 (GraphPad Software Inc., San Diego, USA) was used.

Results

TENM2 is enriched in white adipose tissue progenitor cells

Validation of the previously performed array analysis by qRT-PCR showed that mRNA expression of TENM2 is 6.5-fold higher in white compared to brown adipocyte progenitor cells (Fig 1). Upon adipogenic differentiation of these progenitor cells *in vitro*, TENM2 mRNA expression declined to nearly undetectable levels in cells derived from both depots. Likewise, ZIC1 expression levels were higher in brown compared to white adipocyte progenitor cells, and were further increased after differentiation into adipocytes (Fig. 1B). TENM2 expression in tissue samples was rather low (Supplementary figure S1), comparable to adipocyte samples. Of note, there was a tendency of higher TENM2 expression in white subcutaneous compared to deep neck adipose tissue.

TENM2 expression is decreased upon adipogenic differentiation

To study the regulation of TENM2 expression during adipogenic differentiation we used the human preadipocyte SGBS cell strain. The cells were originally isolated from subcutaneous white adipose tissue of an infant with Simpson-Golabi-Behmel syndrome (Wabitsch et al., 2001) and represent a well-established model for human adipocyte biology (Fischer-Posovszky et al., 2008). Importantly, the model has already been used to study adipocyte browning (Tews et al., 2013). According to the data from primary cells and consistent with the white adipose origin of the cell strain, TENM2 is expressed in undifferentiated SGBS cells to a similar content as primary white adipocyte progenitor cells (0.64+/-0.15 vs. 0.97+/-0.26, Fig 1A and 2). Differentiation into lipid droplet-laden adipocytes (Fig. 2B) leads to marked up-regulation of adipocyte marker genes (PPARG and GLUT4), while TENM2 mRNA expression decreases by approx. 80% at day 14 of differentiation (Fig. 2A).

Knockdown of TENM2 does not interfere with adipogenic differentiation

We decided to take advantage of a siRNA-mediated loss-of-function approach to study the effect of TENM2 on adipogenic differentiation of SGBS cells. Since TENM2 expression is highest in undifferentiated cells and declines with differentiation, SGBS cells were treated with siRNAs prior to adipogenic induction.

Treatment of SGBS preadipocytes with two different siRNAs against TENM2 resulted in a reduction of its mRNA expression by 68 % with construct 1 and by 53 % with construct 2. (Fig. 3A). Bulk cultures of these cells were subjected to adipogenic differentiation. Both TENM2 knockdown and control cells displayed a comparable rate of adipogenic differentiation (Fig. 3B) and lipid content (Fig. 3C). Of note, the knockdown cells appeared to have larger, but fewer lipid droplets (Fig. 3D). However, the mRNA expression of the adipogenic master regulator PPARG and genes, which regulate lipid breakdown and storage (ATGL, HSL, PLIN, CIDEC, ACAA1, ACACA, ACOX1) was not significantly changed between knockdown and control cells (Fig. 3E).

Knockdown of TENM2 induces brown adipocyte marker expression and function

The differential expression of TENM2 in brown and white adipocyte progenitor cells indicates that TENM2 might be involved in the differentiation of preadipocytes towards a white adipocyte phenotype. Thus, we analyzed whether TENM2 deficiency leads to an induction of a brown adipocyte phenotype. Indeed, expression of the brown marker gene UCP1 was increased in adipocytes differentiated from TENM2 deficient SGBS cells (siRNA1: 1.7 fold, siRNA2: 3.1 fold) compared to control cells (Fig. 4A). On the protein level, UCP1 was even stronger induced in TENM2 knockdown cells (siRNA1: 3.1 fold, siRNA2: 4.6 fold, Fig. 4B). The other genes of the UCP family were unchanged. There was a trend towards a higher PGC1a, ELOVL3, CIDEA and CPT1B expression in TENM2 knockdown cells, whereas leptin expression was not altered (Fig. 4A).

During browning of white adipocytes, one might expect an up-regulation of mitochondrial mass and/or mitochondrial activity. Thus, we analyzed both expression of mitochondrial respiratory chain

proteins and citric acid synthase activity. However, both expression of OXPHOS proteins (Fig 4B) and citric acid synthase activity (Fig 4C) were not significantly different between control and TENM2 knockdown adipocytes.

To address how TENM2 deficient adipocytes respond to an adrenergic stimulus, cells were treated with dibutyryl-cAMP to mimic adrenergic stimulation. mRNA data demonstrate that UCP1 expression can be further induced in control as well as knockdown cells (Fig. 4D). Still, UCP1 expression was higher in TENM2 knockdown cells compared to control cells (siTENM2.1: 2.4-fold, siTENM2.2: 6-fold), indicating that the effect of TENM2 knockdown is independent from adrenergic stimulation.

To test whether enhanced UCP1 expression by TENM2 deficiency has further functional impact, we analyzed respiration of TENM2 knockdown and control SGBS adipocytes. Indeed, TENM2 knockdown led to an increase in mitochondrial respiration (Fig. 5A). This resulted in increased both basal and cAMP-stimulated leak respiration rates, pointing to an overall enhanced oxidative metabolism (Fig. 5B).

Discussion

In order to dissect origins of human brown and white adipocytes, we previously analyzed expression patterns of white and brown adipose tissue-derived progenitor cells (Tews et al., 2014). Thus, we identified factors which might predispose adipocyte progenitor cells to differentiate into brown or white adipocytes. One of the factors identified by this approach was Teneurin-2 (TENM2), being 7-fold higher expressed in white compared to brown adipocyte progenitor cells (Tews et al., 2014).

In the present study we were able to demonstrate a causal relationship between the presence of TENM2 and induction of UCP1 and related brown adipocyte marker genes upon adipogenic differentiation in human adipocytes. Thus, our data suggests that TENM2 functions in maintaining a white adipocyte phenotype.

The molecular function of TENM2 is only partially understood, but its structure implicate several mode of actions which might also play a role in adipose tissue. Containing a transmembrane domain (TMR) and several EGF sites at the C-terminal domain indicate that TENM2 is functioning as a cell surface receptor (Vysokov et al., 2016). Thus, TENM2 might receive signals from other cells by cell-cell contact or by binding of specific ligands. Moreover, TENM2 can be proteolytically cleaved at different positions leading to extra- and intracellular fragments (Bagutti et al., 2003; Qian et al., 2004; Tucker et al., 2007). Thus, TENM2 might also act as a soluble ligand or as an intracellular signal transducer.

Indeed, simultaneous transfection of TENM2 lines expressing the nuclear transcription factor ZIC1 led to inhibition of its transcriptional activity (Bagutti et al., 2003). Interestingly, ZIC1 is described as highly expressed in brown adipose tissue and has been frequently used as a molecular marker of classical brown adipocytes (Jespersen et al., 2013; Lidell et al., 2013). Therefore it seems possible that the absence of TENM2 could enhance the activity of ZIC1 and therefore promote a brown

adipocyte phenotype. Although ZIC1 is often used as a marker gene for brown adipocytes, its actual function in adipocytes is currently unknown. ZICs represent zinc-finger proteins, which have an essential role in vertebrate embryonal development (Merzdorf, 2007). For example, the play a role in left-right axis patterning and development of neuronal tissues and the neural crest (Merzdorf, 2007). Interestingly, Zic1 and Zic2 are involved in the regulation of Myf5, the master regulator of myogenesis (Pan et al., 2011) and also a key factor for the determination of white and brown adipogenesis (Enerback, 2009; Seale et al., 2008). This suggests a potential link between TENM2 and brown adipogenesis via ZIC1 and Myf5.

Using two different human *in vitro* systems, SGBS cells as well as primary preadipocytes, we demonstrated, that TENM2 mRNA is expressed in adipocyte progenitor cells and is down-regulated upon adipogenic differentiation. This suggests that TENM2 is involved in differentiation processes or in maintaining a preadipocyte phenotype. To study the role of TENM2 during adipogenic differentiation, we analyzed differentiation of TENM2-knockdown SGBS preadipocytes using siRNA oligonucleotides. SGBS cells had been used for studying adipocyte browning *in vitro* (Guennoun et al., 2015; Tews et al., 2013) and showed TENM2 expression levels comparable to white adipocyte progenitor cells. Therefore, this cell strain provides a suitable model system to study the role of TENM2 in white adipocyte browning *in vitro*. Down-regulation of TENM2 in SGBS cells did not affect adipogenic differentiation rates nor adipogenic marker gene expression compared to control cells. Interestingly, we found alterations in lipid droplet morphology upon TENM2 knockdown besides unaltered triglyceride accumulation, suggesting lipid droplet remodeling. Interestingly, white adipocytes undergo lipid droplet remodeling upon adipocyte browning in mice (Barneda et al., 2013). Such alterations however have not been investigated in human adipocyte *in vitro* cultures so far.

In accordance with our hypothesis that TENM2 is involved in adipocyte browning, the mRNA expression of brown marker genes (UCP1, CIDEA, CPT1B) was induced after differentiation of TENM2 deficient cells, paralleled by increased UPC1 protein levels. Of note though, the up-regulation of brown adipocyte markers was not accompanied by a general increase in mitochondrial mass, as

citrate acid synthase activity and expression of OXPHOS subunits was comparable between knockdown and control cells. This suggests that the UCP1 protein abundance in relation to mitochondrial mass is increased, which is consistent with previous data showing that deficiency of the fat mass and obesity (FTO) gene leads to UCP1 upregulation without induction of mitochondrial mass in the same cell strain (Tews et al., 2013). Primary adipose stromal cells treated with polyunsaturated fatty acids also exhibit an up-regulation of UCP1 without strong induction of mitochondrial mass (Fleckenstein-Elsen et al., 2016). This might also explain why basal and leak respiration rates of TENM2 knockdown cells compared to controls were increased, but only to a moderate extent.

Taken together, we demonstrate that TENM2 is involved in the regulation of UCP1, the primary marker of brown adipose tissue. Knockdown of TENM2 in preadipocytes led to increased brown adipocyte marker expression levels upon differentiation resulting in enhanced mitochondrial respiration. The high expression of TENM2 in white adipocyte progenitor cells suggests that TENM2 functions in maintaining a white adipocyte phenotype during adipogenic differentiation. Inhibition of TENM2 signaling might therefore lead to pharmacologic strategies targeting brown adipose tissue function.

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1 **Tables**

2 **Table 1: siRNA used for TENM2 knockdown**

name	passenger strand (5'-3')
non template control (NT)	GGGGGUACUUUUGUGUAGUACAA
siTENM2.1	GGGGGCAGUGGGAAUGGACAAUA
siTENM2.2	GGGGGUGGGUGACUUCAAUUACA

3

Figure legends

Figure 1: TENM2 expression in white and brown adipocyte progenitor cells and *in vitro* differentiated adipocytes. Progenitor cells from deep neck (dn) and subcutaneous (sc) white adipose tissue were isolated and subjected to adipogenic differentiation and mRNA expression of TENM2 (A) and ZIC1 (B) was investigated by qPCR. Expression values were related to HPRT using the ΔΔCT method. Mean values + SEM of n=12 paired patient samples are shown. **p<0.01, ANOVA.

Figure 2: mRNA expression of TENM2 during adipogenesis in SGBS cells. A mRNA expression of TENM2, UCP1 and adipocyte marker genes at different time points during the course of adipogenic differentiation was analyzed by qRT-PCR. Mean values + SEM of n=4 samples per time point are shown. **B** At different time point during adipogenic differentiation, SGBS cells were stained using OilRed O. Bars represent 100 µM, inlets 5-fold enlarged. **p<0.01, ANOVA.

Figure 3: siRNA knockdown of TENM2 does not interfere with adipogenic differentiation. SGBS preadipocytes were treated with siRNA against TENM2 or control siRNAs 48 hours prior to induction of differentiation. Knockdown of TENM2 was analyzed by qRT-PCR 48 hours after transfection (A). The rate of adipogenic differentiation was determined microscopically (B) and by measuring intracellular triglyceride content 14 days after differentiation start (C). Photomicrographs of day 14 adipocytes (D). Expression of key enzymes involved in adipogenic differentiation and lipid droplet formation were analyzed by qRT-PCR, fold change relative to control cells is shown. Mean values +SEM of 5 experiments are shown, *p<0.05, ***p<0.001, t-test.

Figure 4: siRNA knockdown of TENM2 leads to induction of UCP1 in SGBS adipocytes. SGBS preadipocytes were treated with siRNA 48 hours prior to induction of differentiation. **A** Expression of UCP1,2 and 3 and markers of brown adipocytes was determined by qRT-PCR in control and TENM2 deficient day 14 adipocytes. Mean data +SEM of n=3-5 experiments are shown, *p<0.5, t-test. **B** Expression of UCP1 and OXPHOS proteins in control and TENM2 deficient cells at day 0 and day 14 of adipogenic differentiation was analyzed by Western Blot, one representative blot of three is shown.

med medium control, LF2K lipofectamine control, NT non-targeting control. **C** Specific activity of citric acid synthase was determined in total protein lysates of TENM2 knockdown and control adipocytes, mean+SEM of three independent experiments is shown. **D** Control and TENM2 knockdown SGBS cells were treated at day 13 of differentiation with 0.5 mM dibutyryl-cAMP (cAMP) for 24 hours. mRNA expression of three independent experiments is shown. */*** significantly different from cAMP-treated control cells, *p<0.05, ***p<0.001, ANOVA.

Figure 5: Mitochondrial respiration in TENM2 knockdown adipocytes. A SGBS preadipocytes treated with control or TENM2-directed siRNA were differentiated into adipocytes. Oxygen consumption rates (OCR) were determined in a XFe96 Flux Analyzer (Agilent Technologies) and were plotted against time. To assess basal, leak and maximal respiration rates, cells were consecutively treated with dibutyryl-cAMP (cAMP), oligomycin (oligo), FCCP and antimycin A/Rotenone (Am/R). Representative data of one experiment out of four is shown. **B** Basal, leak and maximal respiration rates were calculated after normalization to values of control cells. Mean +SEM of four experiments is shown, *p<0.05, ANOVA.

References

Bagutti, C., Forro, G., Ferralli, J., Rubin, B., Chiquet-Ehrismann, R., 2003. The intracellular domain of teneurin-2 has a nuclear function and represses zic-1-mediated transcription. J. Cell Sci. 116, 2957–2966. doi:10.1242/jcs.00603

Barneda, D., Frontini, A., Cinti, S., Christian, M., 2013. Dynamic changes in lipid droplet-associated proteins in the “browning” of white adipose tissues. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1831, 924–933. doi:10.1016/j.bbalip.2013.01.015

Bartelt, A., Heeren, J., 2014. Adipose tissue browning and metabolic health. Nat. Rev. Endocrinol. 10, 24–36. doi:10.1038/nrendo.2013.204

Cypess, A.M., Kahn, C.R., 2010. Brown fat as a therapy for obesity and diabetes. Curr. Opin. Endocrinol. Diabetes. Obes. 17, 143–149. doi:10.1097/MED.0b013e328337a81f

Enerback, S., 2009. The origins of brown adipose tissue. N Engl J Med 360, 2021–2023. doi:360/19/2021 [pii] 10.1056/NEJMcibr0809610

Fischer-Posovszky, P., Newell, F.S., Wabitsch, M., Tornqvist, H.E., 2008. Human {SGBS} Cells - a Unique Tool for Studies of Human Fat Cell Biology. Obes. Facts Eur. J. Obes. 1, 184–189. doi:10.1159/000145784

Fleckenstein-Elsen, M., Dinnies, D., Jelenik, T., Roden, M., Romacho, T., Eckel, J., 2016. Eicosapentaenoic acid and arachidonic acid differentially regulate adipogenesis, acquisition of a brite phenotype and mitochondrial function in primary human adipocytes. Mol. Nutr. Food Res. doi:10.1002/mnfr.201500892

Guennoun, A., Kazantzis, M., Thomas, R., Wabitsch, M., Tews, D., Seetharama Sastry, K., Abdelkarim, M., Zilberfarb, V., Strosberg, A.D., Chouchane, L., 2015. Comprehensive molecular characterization of human adipocytes reveals a transient brown phenotype. J. Transl. Med. 13, 135. doi:10.1186/s12967-015-0480-6

Jespersen, N.Z., Larsen, T.J., Peijs, L., Daugaard, S., Homøe, P., Loft, A., de Jong, J., Mathur, N., Cannon, B., Nedergaard, J., Pedersen, B.K., Møller, K., Scheele, C., 2013. A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. Cell Metab. 17, 798–805. doi:10.1016/j.cmet.2013.04.011

Lidell, M.E., Betz, M.J., Dahlqvist Leinhard, O., Heglind, M., Elander, L., Slawik, M., Mussack, T., Nilsson, D., Romu, T., Nuutila, P., Virtanen, K.A., Beuschlein, F., Persson, A., Borga, M., Enerbäck, S., 2013. Evidence for two types of brown adipose tissue in humans. Nat. Med. 19, 631–634. doi:10.1038/nm.3017

Merzdorf, C.S., 2007. Emerging roles for zic genes in early development. Dev. Dyn. 236, 922–40. doi:10.1002/dvdy.21098

Mosca, T.J., 2015. On the Teneurin track: a new synaptic organization molecule emerges. Front. Cell. Neurosci. 9, 204. doi:10.3389/fncel.2015.00204

Pan, H., Gustafsson, M., Aruga, J., Tiedken, J., Chen, J., Emerson, C.J., 2011. A role for Zic1 and Zic2 in Myf5 regulation and somite myogenesis. Dev Biol 35, 120–7. doi:10.1016/j.ydbio.2010.12.037

Qian, X., Barsyte-Lovejoy, D., Wang, L., Chewpoy, B., Gautam, N., Al Chawaf, A., Lovejoy, D.A., 2004. Cloning and characterization of teneurin C-terminus associated peptide (TCAP)-3 from the hypothalamus of an adult rainbow trout (Oncorhynchus mykiss). Gen. Comp. Endocrinol. 137, 205–16. doi:10.1016/j.ygcen.2004.02.007

Rosenwald, M., Perdikari, A., Rülicke, T., Wolfrum, C., 2013. Bi-directional interconversion of brite and white adipocytes. Nat. Cell Biol. 15, 659–67. doi:10.1038/ncb2740

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scimè, A., Devarakonda, S., Conroe, H.M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. a, Beier, D.R., Spiegelman, B.M., 2008. PRDM16 controls a brown fat/skeletal muscle switch. Nature 454, 961–967. doi:10.1038/nature07182

Silva, J.-P., Lelianova, V.G., Ermolyuk, Y.S., Vysokov, N., Hitchen, P.G., Berninghausen, O., Rahman, M.A., Zangrandi, A., Fidalgo, S., Tonevitsky, A.G., Dell, A., Volynski, K.E., Ushkaryov, Y.A., 2011. Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities. Proc. Natl. Acad. Sci. U. S. A. 108, 12113–8. doi:10.1073/pnas.1019434108

Tews, D., Fischer-Posovszky, P., Fromme, T., Klingenspor, M., Fischer, J., Rüther, U., Marienfeld, R., Barth, T.F., Möller, P., Debatin, K.M., Wabitsch, M., 2013. FTO Deficiency Induces UCP-1 Expression and Mitochondrial Uncoupling in Adipocytes. Endocrinology 154, 3141–3151. doi:10.1210/en.2012-1873

Tews, D., Schwar, V., Scheithauer, M., Weber, T., Fromme, T., Klingenspor, M., Barth, T.F., Möller, P., Holzmann, K., Debatin, K.M., Fischer-Posovszky, P., Wabitsch, M., 2014. Comparative gene array analysis of progenitor cells from human paired deep neck and subcutaneous adipose tissue. Mol. Cell. Endocrinol. 395, 41–50. doi:10.1016/j.mce.2014.07.011

Tews, D., Wabitsch, M., 2011. Renaissance of Brown Adipose Tissue. Horm. Res. Paediatr. doi:10.1159/000324806

Tucker, R.P., Kenzelmann, D., Trzebiatowska, A., Chiquet-Ehrismann, R., 2007. Teneurins: transmembrane proteins with fundamental roles in development. Int. J. Biochem. Cell Biol. 39, 292–7. doi:10.1016/j.biocel.2006.09.012

Vysokov, N. V., Silva, J.-P., Lelianova, V.G., Ho, C., Djamgoz, M.B., Tonevitsky, A.G., Ushkaryov, Y.A., 2016. The Mechanism of Regulated Release of Lasso/Teneurin-2. Front. Mol. Neurosci. 9, 59. doi:10.3389/fnmol.2016.00059

Wabitsch, M., Brenner, R.E., Melzner, I., Braun, M., Möller, P., Heinze, E., Debatin, K.M., Hauner, H., 2001. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. Int. J. Obes. Relat. Metab. Disord. 25, 8–15. doi:10.1038/sj.ijo.0801520

Wang, Q. a, Tao, C., Gupta, R.K., Scherer, P.E., 2013. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat. Med. 19, 1338–44. doi:10.1038/nm.3324

Wu, J., Boström, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.-H.H., Khandekar, M., Virtanen, K. a., Nuutila, P., Schaart, G., Huang, K., Tu, H., Van Marken Lichtenbelt, W.D., Hoeks, J., Enerbäck, S., Schrauwen, P., Spiegelman, B.M., 2012. Beige Adipocytes Are a Distinct Type of Thermogenic Fat Cell in Mouse and Human. Cell 150, 366–376. doi:10.1016/j.cell.2012.05.016

Highlights

- TENM2 is higher expressed in human white compared to brown preadipocytes
- TENM2 deficiency in human preadipocytes leads to upregulation of UCP1 expression
- This in turn results in increased basal and leak respiration rates

Figure 1

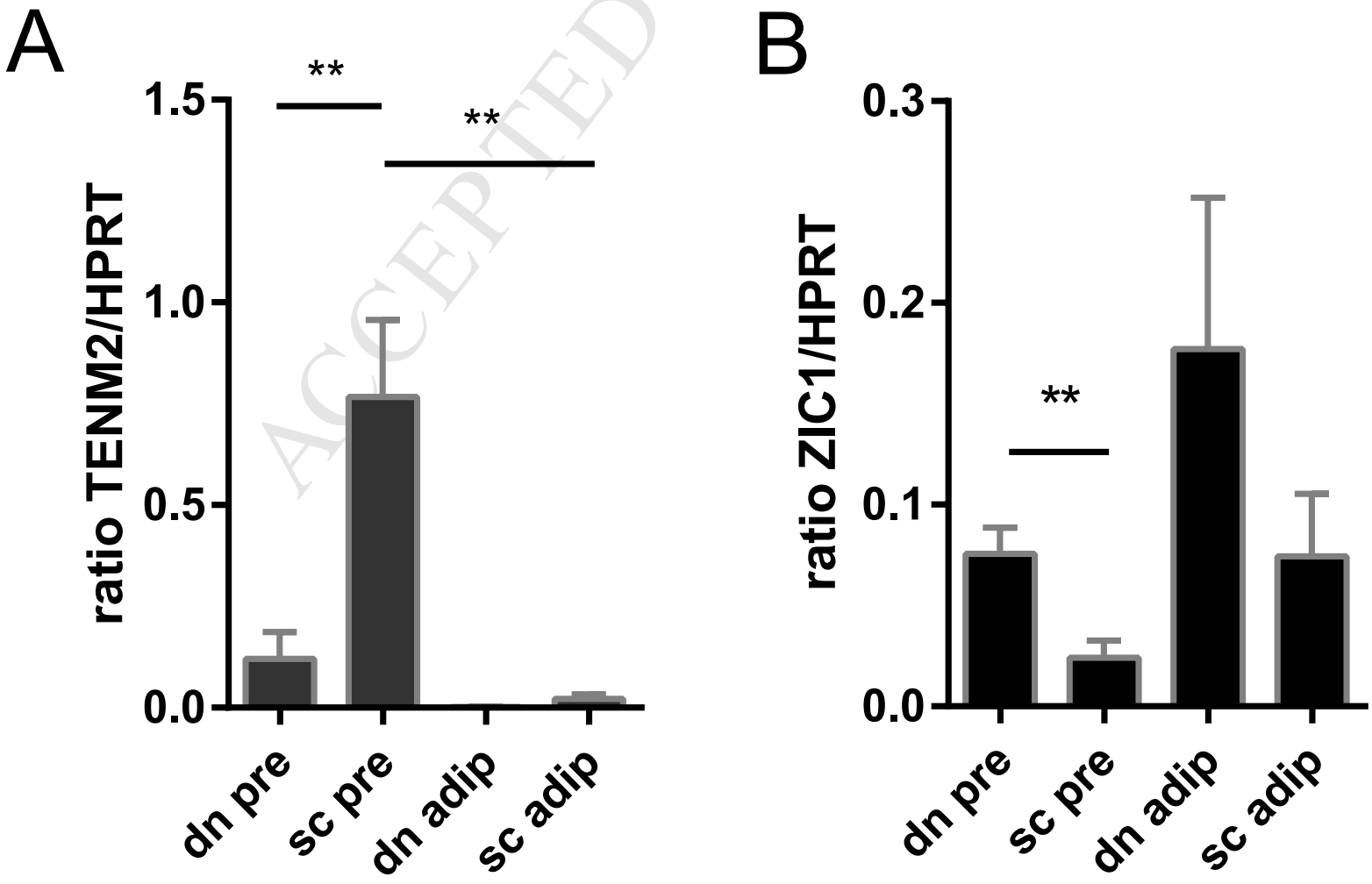
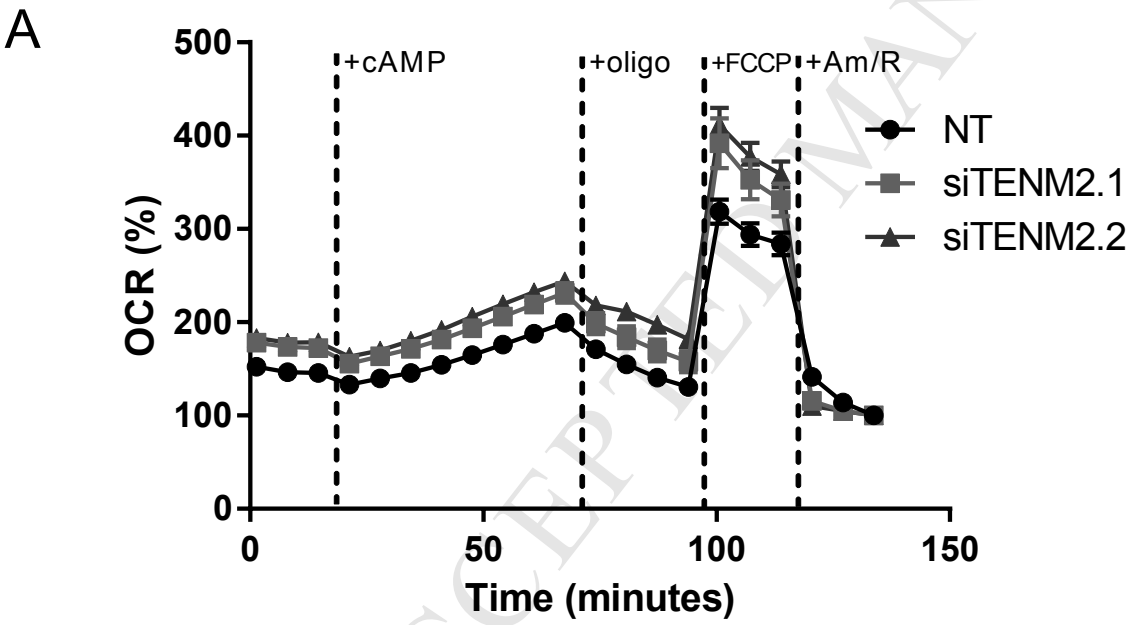


Figure 5



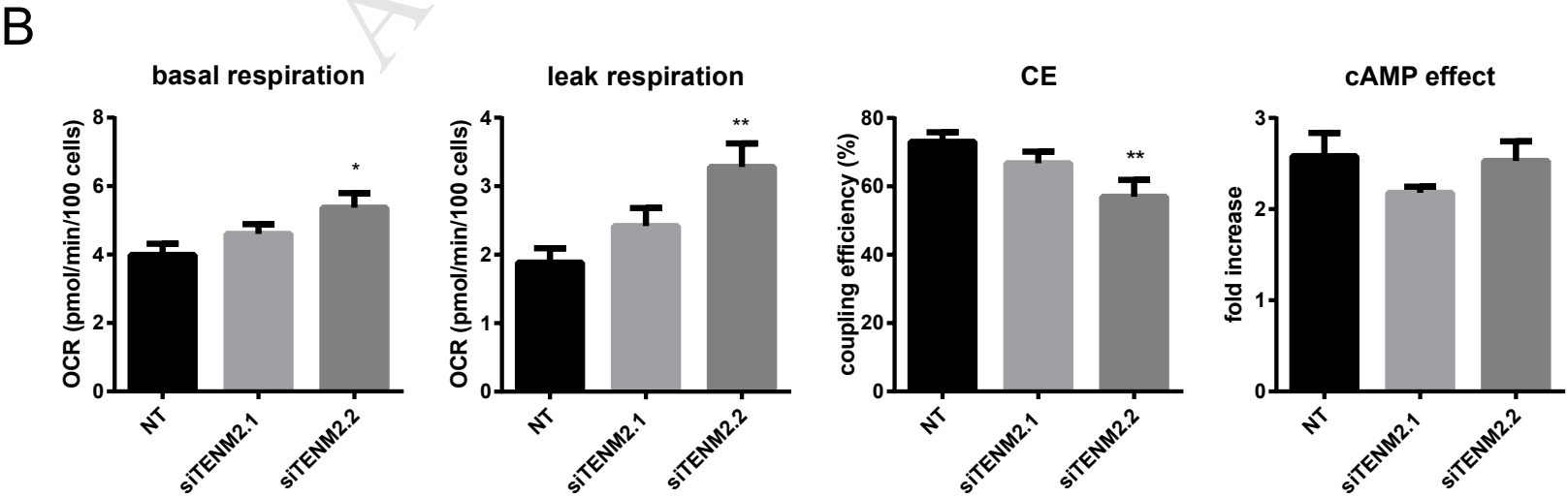
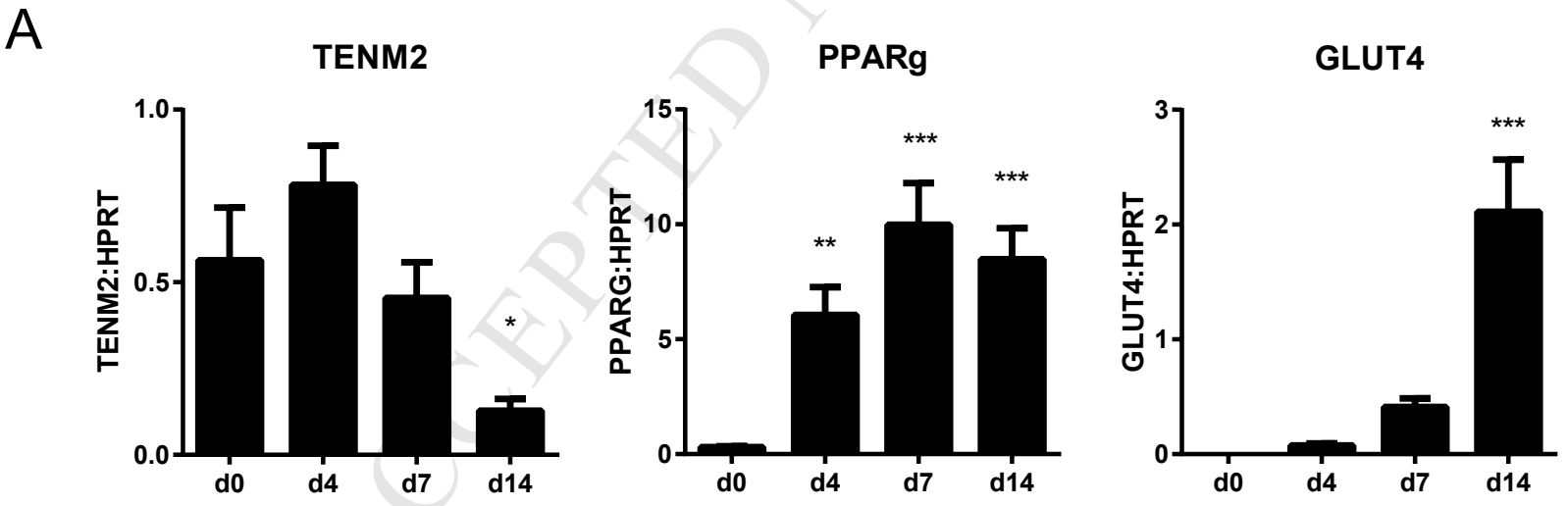


Figure 2



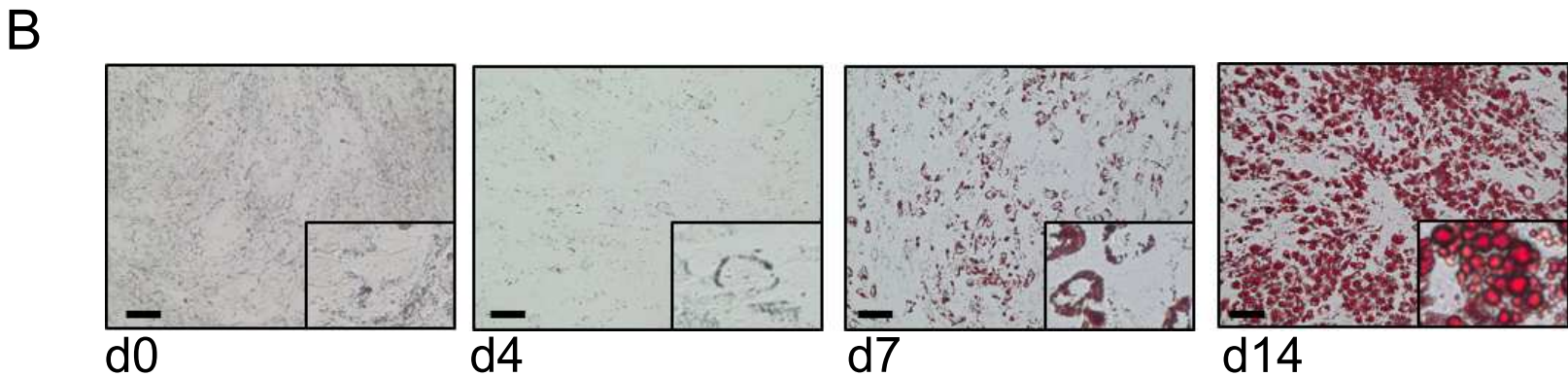
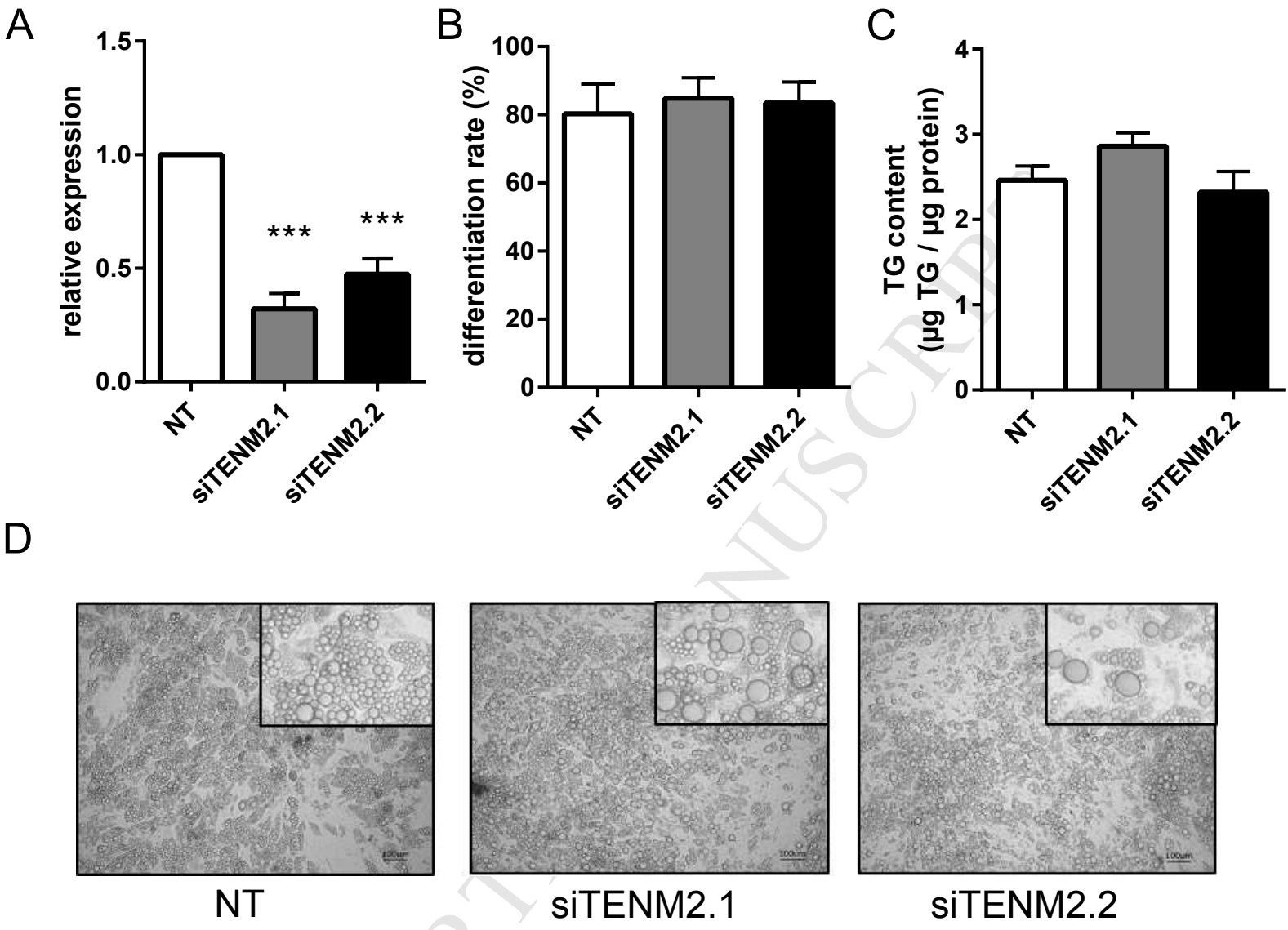


Figure 3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relative expression
1.5
1.0
0.5
0.0
***
***
NT
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siTENM2.2
B
differentiation rate (%)
100
80
60
40
20
0
NT
siTENM2.1
siTENM2.2
C
TG content
(μg TG / μg protein)
4
3
2
1
0
NT
siTENM2.1
siTENM2.2
D
NT
siTENM2.1
siTENM2.2
E
relative expression
2.0
1.5
1.0
0.5
0.0
siTENM2.1
siTENM2.2
PPARg
GLUT4
ATGL
HSL
PLIN
CIDEC
ACOX1
ACAA1
ACACA